THE MAIN STEROID ALCOHOL FROM THE PACIFIC OCEAN HOLOTHURIAN Cucumaria fraudatrix

N. I. Kalinovskaya, T. A. Kuznetsova,
Sh. Sh. Afiyatullov, and G. B. Elyakov

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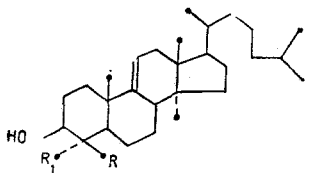
A steroid compound has been isolated from the holothurian Cucumaria fraudatrix. On the basis of ^1H and ^{13}C NMR spectra its structure has been established as 4α,14α-dimethylcholest-9(11)-en-3β-ol.

It has been established previously that marine invertebrates – holothurians – contain complex mixtures of saturated or 7-monounsaturated steroid alcohols with various degrees of alkylation in the side chain [1-8]. In addition to this, we have shown that the Pacific Ocean holothurian Cucumaria japonica [5] has an unusual product of sterol biosynthesis containing an additional α-methyl group at the C_{14} carbon atom and a 9(11)-double bond is the steroid nucleus (sterol I). On studying the sterol composition of the holothurian Cucumaria fraudatrix (Echinodermata, Holothurioidea, Cucumariidae) we have shown that the main sterol in it is also an unusual C_{29}-sterol.

According to its mass spectrum, the new sterol (II) has a molecular ion with m/z 414, which corresponds to a C_{29}-monounsaturated steroid alcohol. Its fragmentation was similar to the mass-spectrometric fragmentation of the 14α-methylcholest-9(11)-en-3β-ol from C. japonica [5] and showed the presence of two additional methyl groups in the steroid nucleus. The C_{14} position of one of these methyl groups was determined by the characteristic strongest peak in the mass spectrum of (II) at m/z 399 ($M^+ - 15$, 100%) [9] and by ^1H and ^{13}C NMR spectra (Tables 1 and 2). The second additional methyl group in the steroid nucleus may, according to the biogenetic prerequisites, be located at the C_4 carbon atom in the α- or β-position. By comparing the ^1H NMR spectra of sterols (I) of one additional doublet with J = 6.25 Hz, assigned to the protons of a methyl group at C_4 [10]. The observed shifts of the signals of the C_3, C_5, C_6, and C_{19} carbon atoms in the ^{13}C NMR spectrum of sterol (II) as compared with their positions in the spectrum of sterol (I) likewise confirmed the presence of a methyl group at C_4 in (II) [11, 12].



I. $R=R_1=H$

II. $R=H$, $R_1=CH_3$

Structure of sterols (I) from C. japonica and (II) from C. fraudatrix

The determination of the configuration at C_4 in sterol (II) can be made on the basis of the ^1H and ^{13}C NMR spectra (Tables 1 and 2). According to the literature, the signal of an axial 3α proton is shifted downfield in the spectra of the 4β-methyl isomers (3.75 ppm) as compared with its position for the 4α-methyl isomers (3.15 ppm) [13]. Moreover, the spin–spin coupling constant for the protons of a 4β-methyl group is larger (8.0 Hz) that for the protons of the 4α- isomer (6.0 Hz) [13].

In the ^1H NMR spectrum of sterol (II) the 3α-proton gives a signal in the form of a characteristic multiplet (d,d,d) occupying a position at 3.03-3.15 ppm; i.e., it is present in a

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TABLE 1. ^1H NMR Spectra of Sterols (I) and (II) (Bruker WH-250 spectrometer; solvent $CDCl_3$; δ, TMS = 0)

Sterol	18-CH_3	19-CH_3	21-CH_3	26-CH_3	27-CH_3	32-CH_3	30-CH_3	H_3	H_{11}
II	0,656 s	0,988 s	0,876 d J=6,25 Hz	0,864 d J=6,30 Hz	0,869 d J=6,35 Hz	0,740 s	0,980 d J=6,25 Hz	3,09 1H d,d,d	5,30 1H,m
I	0,658 s	0,964 s	0,877 d J=6,25 Hz	0,865 d J=6,30 Hz	0,870 d J=6,35 Hz	0,750 s	—	3,59 1H, m	5,29 1H,m

TABLE 2. ^{13}C NMR Spectra of Sterols (I) and (II) (Bruker HX-90 spectrometer; solvent $CDCl_3$; δ, TMS = 0)

C-atom	I	II	C-atom	I	II	C-atom	I	II
C-1	35,5	35,5	C-11	116,4	116 3	C-21	18,4	18,5
C-2	31,6	31,2	C-12	37,3	37,4	C-22	36,5	36,6
C-3	71,2	76,5	C-13	44,3	44,,3	C-23	24,1	24,2
C-4	38,4	39,5	C-14	47,1	47,1	C-24	39,5	39,5
C-5	43,0	49 4	C-15	33,9	33,9	C-25	28,0	28,0
C-6	28,6	24,2	C-16	28,0	28,1	C-26	22,8	22,9
C-7	27,2	27,5	C-17	51,1	51,1	C-27	22,5	26,6
C-8	41,8	41,5	C-18	14,4	14,5	C-30	—	15,3
C-9	145,8	146,4	C-19	19,3	20,5	C-32	18,4	18,5
C-10	38,1	38,7	C-20	36,1	36,2			

stronger field than the corresponding signal in the spectrum of sterol (I) (Table 1). The 4α- configuration of the methyl group is (II) is also indicated by the absence of an upfield shift of the C-2 signal in the ^{13}C NMR spectrum of sterol (II) confirmed the presence of a 9(11)-double bond in it (Table 2). On the basis of the facts given above, we have established the structure of sterol (II) as 4α,14α-dimethylcholest-9(11)-en-3β-ol.

The sterol fraction of the holothurian C. fraudatrix also contained sterol (I) which was identified from its spectral characteristics as 14α-methylcholest-9(11)-en-3α-ol, which we have isolated from the holothurian C. japonica [5]. We have established that the holothurian C. fraudatrix contains no appreciable amounts of sterols other than (I) and (II).

When this work was completed, a report appeared in print of the isolation of a sterol similar to (II) from the holothurian C. frondosa [14].

EXPERIMENTAL

GLC-MS analysis was performed on a LKB-9000 spectrometer. ^{13}C and ^1H NMR spectra were obtained on a Bruker spectrometer. The gas–liquid chromatography of the mixture of sterols and their acetates was performed on a Pye-Unicam 104 chromatograph using a glass column with SE-30 (1.5%) as the stationary phase. The temperature of chromatography was 185°C.

Isolation of Sterols (I) and (II). The dried animals were extracted with chloroform, and the extract was evaporated to dryness. The total chloroform extract was separated on silica gel in the hexane–ethyl acetate (9:1 → 6 : 1) system. The acetates of sterols (I) and (II) were separated on silica gel impregnated with silver nitrate (20%) in the hexane–benzene (10 → 50%) system. The melting point of sterol (II) was 154-155°, $[\alpha]_D^{22}$ + 91.7° (c 0.296; methanol).

SUMMARY

A new steroid compound has been isolated from the Pacific Ocean holothurian Cucumaria fraudatrix. On the basis of its ^1H and ^{13}C NMR spectra, its structure has been established as 4α,14α-dimethylcholest-9(11)-en-3α-ol. Sterols (I) and (II) are the main compounds of the sterol fraction of th is holothurian.

LITERATURE CITED

1. G. B. Elyakov, E. I. Kalinovskaya, V. A. Stonik, and T. A. Kuznetsova, Comp. Biochem. Physiol., 65B, 309 (1979).

2. G. B. Elyakov, T. A. Kuznetsova, Carmen Conde, N. I. Kalinovskaya, A. N. Kalinovskii, and O. F. Smetanina, Khim. Prir. Soedin., 799 (1979).
3. N. I. Kalinovskaya, T. A. Kuznetsova, and G. B. Elyakov, Comp. Biochem. Physiol., 74B, 597 (1982).
4. N. I. Kalinovskaya, T. A. Kuznetsova, A. M. Popov, A. S. Antonov, and G. B. Elyakov, Comp. Biochem. Physiol., 76B, 167 (1983).
5. N. I. Kalinovskaya, A. I. Kalinovskii, T. A. Kuznetsova, V. A. Stonik, and G. B. Elyakov, Dokl. Akad. Nauk SSSR, 279, (3), 630 (1984).
6. K. Gupta and P. Scheuer, Tetrahedron, 24, 5831 (1968).
7. L. Goad, J. Rubinstein, and A. Smith, Proc. Roy. Soc., London, B, 180, 223 (1972).
8. P. Voogt and J. Over, Comp. Biochem. Physiol., 45B, 71 (1973).
9. N. Ragsdale, Biochim. Biophys. Acta, 380, 81 (1975).
10. W. Kokke, L. Bohlin, W. Fenical, and C. Djerassi, Phytochem., 21, 881 (1982).
11. H. Eggert and C. Djerassi, J. Org. Chem., 46, 5399 (1981).
12. F. Wehrli and T. Nishida, Prog. Chem. Org. Nat. Prod., 36, 994 (1979).
13. F. Knapp and G. Schropfer, Steroids, 26, 339 (1975).
14. J. A. Findlay, A. Daljeet, J. Matsoukas, and Y. E. Moharir, J. Nat. Prod., 47, 560 (1983).

STEROIDS OF THE SPIROSTAN AND FUROSTAN SERIES FROM PLANTS OF THE GENUS Allium.

XXI. STRUCTURE OF ALLIOSPIROSIDE A AND ALLIOFUROSIDE A FROM Allium cepa

S. D. Kravets, Yu. S. Vollerner,
M. B. Gorovits, A. S. Shashkov,
and N. K. Abubakirov

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Two new steroid glycosides have been isolated from the generative organs (pericarps and peduncles) of Allium cepa L.: alliospiroside A and alliofuroside A. According to chemical transformations and spectral characteristics, alliospiroside A has the structure of (25S)-spirost-5-ene-1β,3β-diol 1-O-[O-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside]. The structure of (25S)-furost-5-ene-1β,3β,22α,26-tetraol 20-O-β-D-glucopyranoside 1-O-[O-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside] has been established for alliofuroside A.

Continuing a study of the steroids of the spirostan and furostan series from plants of the genus Allium [1], we have investigated the generative organs (pericarps and peduncles) of Allium cepa L. (garden onion, Uzbekestan variety "Karatal") after the elimination of the ripened seeds. From the total extractive substances, six steroid glycosides present in In the present paper we describe proofs of the structures of the two glycosides present in the largest amount, which we have called alliospiroside A (I) and alliofuroside A (IIa).

From its positive color reaction with vanillin/phosphoric acid [2] and its characteristic absorption in the IR spectrum [2, 4], compound (I) was assigned to the (25S)-spirostan series.

The hydrolysis of alliospiroside A (I) gave the aglycon (III), the acetylation of which with acetic anhydride in pyridine led to the diacetate (IV). The physicochemical constants and spectral indices of products (III) and (IV) permitted the genin (III) to be identified as (25S)-ruscogenin [5, 6].

The methanolysis of glycoside I followed by analysis of the sugars by GLC [7] showed that the carbohydrate moiety of alliospiroside A (I) included one L-arabinose residue and one L-rhamnose residue.

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